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MAY 16 1949

VOLUME 7 NUMBER 4

NUTRITION REVIEWS

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MAY 2

April 1949

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Published Monthly by

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK, N. Y.

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NUTRITION REVIEWS

VOL. 7 APRIL, 1949 NO. 4

NUTRITION IN ENDOCRINOLOGY

Alterations in glandular function are always accompanied by disturbances in nutrition since each of the major endocrine glands controls at least one specific metabolic function or metabolite. Thus the internal secretion of the pancreas accelerates glucose metabolism, testicular secretions accelerate protein and mineral storage, adrenal cortical secretions influence salt, water, carbohydrate, and protein metabolism, the parathyroids act as regulators of calcium and phosphorus, pituitary growth hormone acts to cause nitrogen retention, the thyroid accelerates cellular metabolic processes, thus affecting each and every nutrient, and the ovaries stimulate calcium and protein retention.

Consequently, the hypo- or hyperfunctioning of an endocrine gland is usually followed by nutritional imbalance. Correction of the defect involves correcting both the hormonal and the nutritional derangements.

Combinations of the hormones with specific dietary regimens are useful in coping with various generalized systemic diseases not directly attributable to the endocrine system. In this review each of the major endocrine problems will be summarized, and the combined use of nutrients and hormones as adjuncts in the treatment of nonendocrine conditions will be mentioned.

Testes. Androgenic failure at puberty not only results in sexual infantilism but leads to two serious nutritional defects: (1) Lack of adequate conversion of amino acids to protein. This prevents normal development of skin (which remains soft and childlike), muscles (which remain poorly developed and lack the usual muscle mass), and bones (which, partially as a result of protein lack, and partially from a negative mineral balance, fail to undergo epiphyseal closure, eventually resulting in eunuchoidal skeleton structure. The bones are then thin and poorly developed, and in later years become definitely osteoporotic and increasingly liable to fracture). (2) Lack of mineral retention, especially negative calcium, phosphorus, sulfate, and potassium balances. This adds to the defect in bone development, repair, and maintenance already impaired by the negative nitrogen balance.

Androgenic failure occurring after maturation is attained, is accompanied by regressive changes in skin, bone, and muscle as well as by loss of masculinity.

Neither substitution therapy using testosterone nor increased intake of the nutrients, amino acids, and minerals, if given alone, will cause a reversal of the changes occasioned by the androgenic failure. Testosterone therapy becomes maximally effective only in the presence of a suitable nutritional substrate—proteins and minerals—and thus provision for administering both must be made.

Ovaries. Estrogenic failure is similarly accompanied by negative nitrogen and mineral balance. Calcium balance is more markedly influenced by estrogens than by androgens. Consequently, severe osteoporosis develops more rapidly in castrated women. The metabolic defects are in other ways similar to those encountered in androgenic failure, and their correction depends upon the use of both nutrients (especially calcium intake) and estrogens.

It should be noted that following castration in either sex, severe metabolic disturbances can be postponed almost indefinitely if intake of proteins and minerals is sufficient. Conversely, if the nutritional status remains marginal or submarginal, the castrated individual develops regressive tissue changes much more rapidly. Similarly, the patient

with marginal or submarginal nutrition whose gonads are secreting adequately will as a general rule not suffer from as severe regressive bone, skin, and muscle changes as the castrated individual on the same diet. One may infer from this that the hormones increase the efficiency of utilization of proteins and minerals, and that in the absence of the hormones, the nutrients, if given in large enough quantity, can (possibly by the law of mass action) in part overcome the hormonal deficiency in so far as the metabolic defects of such a deficiency are concerned.

Adrenals. In complete adrenal insufficiency (Addison's disease), negative water, sodium chloride, and protein balance is accompanied by excessive retention of potassium and failure of conversion of amino acids to glucose leading to hypoglycemia.

The primary defect in Addison's disease leading to water and electrolyte disturbances is renal. The kidneys are able to concentrate or dilute urine within rather narrow limits only, and are unable to retain sodium ions but are able to retain potassium ions. Potassium is retained in greater than normal amounts leading to hyperpotassiumemia and, since chloride accompanied the sodium, a deficit in bodily salt occurs. The sodium chloride deficiency and water loss results in decreasing blood volume and reduced blood pressure, and leads to shock. Control of this chain of events may be achieved in part by hormonal therapy (the sodium- and water-retaining hormone, desoxycorticosterone acetate), and in part by administering the nutrients salt and water. In complete adrenal failure, neither, alone, will serve to correct the deficit; but when given together, they quickly restore the body to normalcy.

A patient with Addison's disease also lacks "S" hormones, the adrenocortical steroids which allow conversion of amino acids to glucose. Carbohydrate metabolism *per se* is only slightly affected; but if carbohydrate intake is poor, then, since glycogen storage is decreased in the absence of "S" hormones, hypoglycemia and coma may quickly ensue. Therefore, an integral part of the successful treatment of Addison's disease is the provision for multiple small carbohydrate feedings to maintain a steady source of carbohydrate between meals. The amount of carbohydrate must be increased following any extraordinary muscular effort, to supply additional glucose. Patients with Addison's disease, like diabetic patients, should always carry candy or sugar lumps to prevent attacks of hypoglycemia.

In Addison's disease, the "N" nitrogen-retaining hormone of the adrenal is absent leading to negative nitrogen balance, loss of muscle mass and strength, weight loss, and asthenia. This may be corrected by using either whole adrenal extract, which contains all the adrenal hormones but is very expensive, or by supplementing with testosterone or estrogens. An adequate protein diet is of course mandatory.

Thyroid. Thyroid hormone is one of the principal regulators of the metabolic processes of the body, stimulating metabolism in all cells. In hypofunction, slower metabolism results in a lowered basal body temperature, decreased pulse rate, and a decreased turnover of proteins, carbohydrates, fats, minerals, and vitamins. If an individual suffering from hypothyroidism maintains a normal caloric intake, the excess calories will be stored as fat. Treatment with thyroid increases utilization of food as energy. To maintain a constant weight, an increased food intake is necessary. This requirement is usually automatically satisfied by an increased appetite.

In hyperthyroidism, all cellular processes seem to be accelerated, causing rapid depletion of all nutritional elements. Thus fat stores are quickly exhausted; the patient loses weight and becomes gaunt, protein utilization is hastened resulting in protein loss from all tissues, which causes further weight loss in the patient and is reflected most strikingly in loss of muscle mass.

Demand for carbohydrate is excessive, glycogen stores become depleted, and hypoglycemia often results; mineral turnover is greater, and this is commonly reflected by thinning of bones and fractures. Vitamin utilization is increased resulting in overt niacin, thiamine, and riboflavin deficiency in the average case. In addition, other essential nutritional elements such as choline, inositol, and certain amino acids are exhausted, causing fatty livers and followed often in long-standing cases by cirrhosis and jaundice.

Indispensable for successful therapy is correction of the nutritional deficiency regardless of the specific treatment applied to the thyroid gland, i.e., surgical removal, antithyroid drugs, or radioactive iodine.

Parathyroids. In dysfunction of the parathyroids, a specific disruption of mineral metabolism is effected through the primary action of the hormone of the parathyroids to stimulate renal excretion of phosphorus. In hypofunction, decrease in urinary excretion of phosphorus causes an increase in serum phosphorus and a reciprocal drop in serum calcium to levels which cause tetany. The only dietary indication here is administration of large amounts of calcium in the form of lactate or gluconate, administered at repeated intervals during the day. Hyperparathyroidism, which is characterized by a hypercalcemia with renal stones, calls for surgical removal of the hyperfunctioning tissue with no specific dietary requirements.

Pancreas. Dysfunction of certain cells in the pancreas gives rise to diabetes. The importance of nutrition in diabetes will not be taken up here as it has already been thoroughly discussed in this journal (*Nutrition Reviews* **6,** *257 (1948)*).

Pituitary. In hyperfunction, such as results in acromegaly or gigantism, the primary aberration is an overproduction of growth hormone with an attending overgrowth of all tissues due to excessive tissue formation. The needed protein is usually automatically supplied by an increased appetite.

In hypofunction, the resulting asthenia, wasting, or cachexia can be ascribed to secondary failure of the other endocrine glands. Provision of all food elements is an integral part of therapy.

A condition in all respects similar to hypopituitarism may be produced by starvation or malnutrition, such as is encountered in anorexia nervosa. The pituitary, although anatomically intact, fails to release hormones into the blood stream and the other glands fail. Substitution therapy with hypophyseal extracts or thyroid, gonadal, and adrenal cortical substances is of no benefit unless the nutritional deficits are first corrected, and if rapidly corrected, administered hormones may not be essential for recovery.

Nonendocrine diseases in which hormones and nutrients are important adjuncts in the therapeutic program have been discussed by E. C. Reifenstein and F. Albright (*see Nutrition Reviews* **5,** *249 (1947)*). We should like to reemphasize that the "N" hormones (testosterone is usually used) are without material effect unless protein and mineral intake is adequate.

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AVAILABILITY OF AMINO ACIDS FROM PROTEIN FOODSTUFFS

Just as a steady supply of material is essential for the construction of a new building or for its maintenance in a good state of repair, so a steady stream of nutrients is a prerequisite for the growth and maintenance of the human structure. With the twenty

or more amino acids which compose the proteins of tissues the illustration is particularly pertinent because the body requires an outside source of approximately ten of these noninterchangeable and individual amino acid units. Lysine may be used as an illustration of the indispensable group. When it is lacking, the manufacture of body protein is inhibited, regardless of the presence or absence of other amino acids. Dietary amino acids are not entirely useless if protein formation is retarded by the lack of one building unit, such as lysine. Some may be used as fuel even though their use for this purpose is uneconomical, protein being one of the more expensive foodstuffs. Some might be used in the manufacture of more essential tissue protein if less important protein is disintegrated to procure the lysine which is present, but this is also a wasteful process.

The efficient provision of a steady supply of essential amino acids in the right proportion is not as simple as it sounds. Efficiency implies proper use with minimum waste. Is it possible to adjust one's eating habits so that the presence in the body fluids of no more than a reasonable surplus of each indispensable amino acid is assured at all times? The principal factors which will determine the answer to this question are the following: availability of foodstuffs that will supply in a minimum amount of total protein those amino acids which the body can convert into its own protein; complete digestion of the protein in the alimentary canal; absorption of the liberated amino acids into the blood stream; and, simultaneous presence of sufficient calories from carbohydrate and fat for the energy supply and of other dietary essentials for normal metabolic functions.

Considerable data have accumulated on the digestibility of individual food proteins but in terms of total nitrogen and not in terms of each amino acid. Information on this important point has been reported for a few proteins by K. A. Kuiken and C. M. Lyman (*J. Nutrition* **36,** *359 (1948)*) who have determined the availability of the ten indispensable amino acids in roast beef, cottonseed flour, peanut flour, and wheat. These foodstuffs were incorporated into the rations so as to provide protein at a 10 per cent level. Rats were fed the diets for a seven day period during which time an accurate record was kept of the food intake and of the fecal excretion. Both the food and the feces were analyzed for their content of the ten essential amino acids by microbiologic methods. It was assumed that wastage of amino acids by urinary excretion did not occur because of the relatively low protein intake. Comparison of the fecal excretion of each amino acid during the experimental period with that which occurred during a similar control period on a diet containing 6 per cent of defatted whole egg as the sole source of dietary protein was used as an index of true availability. The assumption was made that the amino acids of egg protein were entirely utilized (*Nutrition Reviews* **5,** *112 (1947)*).

The results with roast beef showed complete availability of each of the ten amino acids. An entirely different result was obtained with the cottonseed flour. There were marked variations in the availability of the amino acids, ranging from 65 per cent for lysine to 94 per cent for arginine. With peanut flour and wheat small losses in the feces occurred but these were uniformly distributed among the various amino acids. In terms of total nitrogen the true availability of the amino acids of peanut flour and wheat amounted to 98 and 95 per cent, respectively. The poor availability of lysine from cottonseed protein was not due to overloading because the total amount in this material was less than one half of the amount present in roast beef protein. The results clearly illustrate the effect of incomplete digestion and absorption on the biologic value of a protein.

The explanation of differences in digestibility with variable losses of individ-

ual amino acids is not known. It may be related to the physical state of the material that is ingested or it may be dependent upon the chemical combinations of the amino acids in the protein molecule. It is entirely possible that lysine in cottonseed meal may occur in peptide structures which under certain circumstances may be resistant to the action of the proteolytic enzymes of the digestive tract. It is known that lysine is adversely affected by the heat processing of foods (*Nutrition Reviews* **5**, *83* (*1947*)).

The experiments of Kuiken and Lyman are important because the fecal nitrogen has been characterized in terms of essential amino acids, not in terms of total nitrogen. The determination of the degree of digestion and of absorption of amino acids of dietary protein is only a part of the measurement of actual biologic value because the absorbed amino acids may or may not resemble the mixture required by tissues. Studies of this type must be combined with studies of the utilization of individual amino acids after their absorption in order to provide the data that will permit one to supply amino acids from dietary protein in the most efficient manner.

EFFECT OF SCHOOL FEEDING PROGRAMS ON NUTRITIONAL STATUS

Child feeding programs have been initiated in many countries as shown in a recent publication of the Food and Agriculture Organization of the United Nations ("National Progress in Food and Agriculture Programs," *Columbia University Press, New York, N. Y.* (*1948*)). While the importance of such programs is generally recognized, few reports of the specific effects of school feeding on nutritional status are available. In 1945, a study of the nutritional condition of children in rural South Carolina in relation to school lunches was reported by A. M. Moser (*S. Carolina Agr. Exp. Sta. Bull.* **359** (*1945*)). Forty-nine children in a school having a partial lunch, and 37 children in a school where a complete lunch was served were followed for one and one-third years. The complete lunch supplied about one third of the average daily requirement for calories and protein and one half of that for minerals and vitamins, while the partial lunch consisted of surplus foods, chiefly dried or fresh fruit. Nutritional status, as evaluated by a pediatrician, improved considerably in children who received the complete lunch while no change occurred in the other group. Children of the complete lunch group made superior gains in height during the period of study, whereas weight gains were essentially the same in the two groups. The complete lunch included more milk and whole grain cereal products than did the partial lunch. A correlation between stature of schoolchildren and milk consumption in the home has been previously reported (F. M. Williams, H. K. Stiebeling, I. G. Swisher, and G. Weiss, *U. S. D. A. Tech. Bull.* **576** (*1937*)). Hemoglobin concentration was maintained more satisfactorily in children receiving the complete, as compared with the partial lunch, which appears to be a significant achievement in view of the demands of growth. Little difference was noted in levels of vitamin A and carotene in the blood between the two groups of children.

A five-year study was made of the effectiveness of a school lunch program in improving the nutrition of rural children in Florida (O. D. Abbott, R. O. Townsend, R. B. French, and C. F. Ahmann, *Florida Agr. Exp. Sta. Bull.* **426** (*1946*)). The lunch supplied at least the minimal daily allowance of essential food factors and for the first four years was under direct supervision of a dietitian. Supplements of iron, vitamin A, or ascorbic acid were given when evidence of severe deficiency of these factors was noted. Prevalent defects in the 186 children studied were dental caries, defective tonsils,

gingivitis (90 per cent), anemia (71 per cent), follicular conjunctivitis (71 per cent), cheilosis and angular stomatitis (64 per cent), and skeletal defects (63 per cent). These findings, obtained in 1940, were compared with those noted in 1942 and 1944. A decrease in the incidence of follicular conjunctivitis, skin defects, gingivitis, and angular stomatitis occurred. The effect of the school lunch on average body build and average developmental age was determined by plotting height, weight, and age data on the Wetzel grid and comparing the basal curve for 1940 with curves for subsequent years. "There was a shift in channels toward better physique and a change in developmental level from a lag to a lead." For the first four years, hemoglobin values increased during the school year and decreased during vacation with a yearly net gain. During the fifth year a net loss occurred. At this time, the school lunch was not being supervised by trained personnel and diets appeared to be inadequate in vitamins A, C, and riboflavin, as well as in factors needed for regeneration of hemoglobin. It was concluded that the school lunch is an effective means of raising nutritional status when adequately supervised, when planned to take care of known deficiencies, and when supplementary vitamins or minerals are given as needed.

C. Urbach, P. B. Mack, and J. Stokes (*Pediatrics* **1,** *70 (1948)*) studied, during two successive years, three approximately matched groups of undernourished elementary schoolchildren (average age, 11 years) who were fed school lunches and enriched cereal, a calorically equivalent amount of common farina, or no cereal. The enriched cereal contained a higher percentage of protein, which was of the predigested type, than the farina, as well as larger amounts of calcium, phosphorus, iron, copper, thiamine, riboflavin, niacin, and pantothenic acid. Both cereals were served in 30 g. quantities (dry weight) daily with 4 ounces of milk. One-week records of home dietaries, obtained at two periods of the study by the school nurse, were stated to be equally poor in the three groups of children, with a decrease in adequacy after the school lunch program was instituted. Medical, dental, x-ray, and biochemical examinations were made initially, and at the end of seven months, by examiners who had no knowledge of the groups to which the children belonged.

Thirty to 33 per cent of the children receiving either of the cereal supplements gained 10 per cent or more in weight between the first and second tests each year, while 14.7 per cent of those receiving no supplement made a similar gain. The group receiving enriched cereal showed greater improvement in a pediatrician's rating of general nutritional status than did the other groups. The percentage of children whose skeletal maturation was similar to that of well nourished children of the same age increased by about 10 per cent during each year in the enriched cereal group, and remained stationary in the other two groups. Skeletal mineralization, as judged by the technic of P. B. Mack and J. M. Smith (*Pa. State College Bull.* **33,** *No. 43 (1939)*), improved slightly in the enriched cereal group and became less satisfactory in the other two groups. An increase in dental caries was noted in the children receiving farina or no cereal, whereas the incidence was unchanged in those receiving enriched cereal.

A biomicroscope was used to examine the eyes, tongue, and gums. No significant change in opacity or translucency of the conjunctiva was observed in any group. Recession of corneal vascularization was reported in a larger percentage of the children who received enriched cereal than in the other children. Improvement in the condition of the tongue and of the gums was noted in all groups but was most marked in the one given enriched cereal. Differences were stated to be statistically significant but no figures were included.

In the laboratory studies, values for hemoglobin, erythrocyte count, and volume of

packed red cells showed no improvement in any group. Phosphatase decreased in the blood in more than 50 per cent of the children receiving cereal and in 38 per cent of those receiving no cereal. The average blood concentration of vitamin A and of carotene showed no significant change during the period of study. However, the authors stated that in each year, the number of children showing a blood level of vitamin A of 30 micrograms per cent increased significantly in the enriched cereal group. Since the enriched cereal contained no vitamin A, the authors suggested that the findings might be related to a sparing action of protein, or possibly of the "B-vitamins," on vitamin A. In the absence of other data, it seems unwise to attach significance to an increase in the blood level of vitamin A above a certain arbitrary figure in these few children. No data are given concerning individual dietary intakes of vitamin A, although it was noted that during the second year of study, diets were higher in vitamin A in the entire group of children than during the first year. An improvement in dark adaptation was found in each of the three experimental groups during each year.

The mean concentration of ascorbic acid in the blood plasma increased in the children receiving enriched cereal, the probability being greater than 100 to 1 that the increase was not due to chance. In this group of children, the number with values for ascorbic acid in the blood of 1.2 mg. per cent also increased. The authors suggested that a protein-ascorbic acid synergy or a relation between thiamine and ascorbic acid might explain the findings. It is unfortunate that data were not included for ascorbic acid in the home diets of children showing elevation in blood levels. More information is certainly needed concerning interrelations of vitamins or of vitamins and protein.

A significant increase in the excretion of riboflavin in a fasting one-hour sample of urine was found in the enriched cereal group, a decrease or no change in the other two groups. An increase in thiamine excretion also occurred in the children receiving enriched cereal. This increase "approached but did not reach statistical significance."

Mean values for total serum protein decreased slightly in all groups during both years while serum albumin levels, measured only in the last year, likewise decreased. There was a reduction in protein in the home dietary during these periods. Plasma vitamin E was measured in the second year of study and showed no change.

The above studies indicate that a school lunch program can be an effective aid in improving nutrition and emphasize the importance of milk and of whole grain or enriched cereals in child feeding.

VITAMIN DEFICIENCIES AND TRYPTOPHAN METABOLISM

The metabolism of tryptophan in the animal body has received considerable attention. It is now known to give rise to urinary kynurenine, kynurenic acid, and xanthurenic acid (*Nutrition Reviews* **3,** *72 (1945)*) and to serve as a precursor from which niacin may be synthesized in the body. Its administration in excess also increases, therefore, the excretion of N′-methylnicotinamide (*Ibid.* **5,** *247 (1947)*) and presumably other metabolites of niacin.

It has been frequently shown that the amounts in which these various products are excreted following ingestion of tryptophan are markedly altered when the animal is deficient in pyridoxine. Under these conditions, the amount of xanthurenic acid in the urine is increased (*Nutrition Reviews* **3,** *343 (1945)*). Pyridoxine also influences the metabolism of this amino acid in other ways, since it is necessary for synthesis of tryptophan by organisms which carry out this

reaction, and is also required for the transformation of tryptophan to niacin.

Because of these relations of pyridoxine to tryptophan, and because the quantitative relations among the various tryptophan metabolites were obscure, C. C. Porter, I. Clark, and R. H. Silber (*Arch. Biochem.* **18,** *339 (1948)*) decided to study the effects of deficiencies in a variety of "B vitamins" upon the metabolism of this amino acid. To this end, adult male rats were fed appropriate diets deficient in thiamine, riboflavin, or pyridoxine until their weights had become static or were decreasing. At this time, groups of the animals received by stomach tube, 230 mg. of L-tryptophan and 1 mg. each of the "B vitamins" in which they were not deficient. After nineteen and a half hours, each rat received 10 ml. of water by stomach tube. Three hours later, the urines were collected and combined with cage washings. Kynurenine, kynurenic acid, and xanthurenic acid were determined on each sample. The quantitative results secured were of interest. The total amount of the three products recovered (in terms of percentage of administered dose of tryptophan) depended upon the diet as follows: normal group, 17 ± 2.4; thiamine deficient, 25 ± 7.9; riboflavin deficient, 43 ± 5.0; pyridoxine deficient, 73 ± 9.8. Combined deficiencies of two or more vitamins gave the effects which would be predicted from consideration of these figures. For example, the highest percentage of the test dose of tryptophan was recovered from animals deficient in both thiamine and pyridoxine, and amounted to 80 ± 8 per cent of the administered tryptophan.

The effects upon the individual metabolites were also of interest. In normal animals, roughly equal quantities of xanthurenic and kynurenic acids were excreted, and each of these was excreted in amounts roughly twice that of kynurenine.

These ratios, as well as the absolute amounts of the products excreted, were markedly changed during vitamin deficiency. The thiamine deficient animals excreted relatively much more xanthurenic acid and much less kynurenic acid; riboflavin deficient animals excreted relatively much more kynurenic acid and much less xanthurenic acid; pyridoxine deficient animals (in accordance with previous results) excreted markedly less kynurenic acid and much more xanthurenic acid than the controls. Although the single most striking result was that obtained during pyridoxine deficiency, the effects obtained during thiamine and riboflavin deficiency were nonetheless significant. Again the effects of combined deficiencies were essentially those which would be predicted from combined results of the single deficiencies.

It may be inferred from these results that all three vitamins are involved in the disposition of the various metabolites of tryptophan. Particularly in riboflavin and pyridoxine deficient animals, the total recovery of L-tryptophan as its known metabolites is increased. This must mean that these products are excreted either because they are not readily metabolized further by the deficient animal, or that their appearance results from abnormal metabolism of the precursor which normally gives rise to them. In either case it indicates that these products are not necessarily "normal" products of tryptophan metabolism at all, and rats receiving ample vitamins and minimum quantities of tryptophan might not excrete any of these products.

The authors interpret an increase in the excretion of either kynurenic acid or xanthurenic acid without a proportionate increase in the other as evidence that the formation of one of the two acids from kynurenine is proceeding at a suboptimum rate. In thiamine and pyridoxine deficiency, a reduced conversion of kynurenine to kynurenic acid is thus indicated; whereas, in riboflavin deficiency a reduced conversion of kynurenine to xanthurenic acid is indicated.

These interesting observations emphasize

the extent to which the intimate details of body chemistry are dependent upon the dietary intake. As observed by these authors, the changes occurred during the mild preliminary phases of vitamin deficiency, before pronounced symptoms of vitamin-lack became apparent. Perhaps it is partially through the accumulation of such abnormal products of metabolism that vitamin-lack, which under conditions normally encountered is never absolute, and which may thus interfere with only a few bodily reactions, contributes to such far reaching pathologic changes in deficient animals.

NUTRITION AND RESISTANCE TO INFECTION

The relation of the plane of nutrition to resistance of an organism to infection has been frequently discussed in these pages (*Nutrition Reviews* **5,** *63, 152 (1947)*). In an investigation of this subject, K. Guggenheim and E. Buechler (*J. Immunology* **58,** *133 (1948)*) have determined the effect of quantitative and qualitative variation in the protein of the diet on the bactericidal properties and phagocytic activity of the peritoneal fluid of rats. Rats were fed diets containing 18, 9, 6, or 3 per cent of casein, with rice starch, olive oil, salt mixture, and vitamins for a period of four weeks, then injected intra-abdominally with 0.5 ml. of a twenty-four hour broth culture of Salmonella typhimurium. Two hours later, the peritoneal fluid was obtained and counts made of leucocytes, phagocytes, and bacteria in the suspension. The number of extracellular bacteria found in the peritoneal fluid itself, and in its supernate increased regularly and markedly as the casein content of the diets decreased, indicating that the bactericidal power of the peritoneal fluid is diminished in protein deficiency. There was also a statistically significant decrease in the number of leucocytes mobilized on the low protein diets. Only in the severest protein deficient ration (3 per cent casein) was there a statistically significant reduction of phagocytes, and even here, the phagocytic index was not lowered.

A comparison of the relative values of proteins of egg, meat, soybean, peanut, and maize in maintaining the bactericidal and phagocytic properties of peritoneal fluid was next made by feeding these proteins as the sole protein of the ration at the 9 per cent level. Again, marked differences were observed. The most effective protein was that of egg, followed in order by meat, casein, soybean, peanut, and maize. Diets containing 9 per cent of egg or meat proteins elicited bactericidal and phagocytic activity roughly equivalent to that obtained on a diet containing 18 per cent of casein; soybean protein and casein were approximately equivalent, whereas 9 per cent of maize protein was similar to 3 per cent of casein. Here, too, the phagocytic mechanism of defense was less affected than the humoral mechanism by variation in the protein.

In both the quantitative and qualitative studies, the number of surviving bacteria was in inverse ratio to the weight increase which the protein source allowed. It therefore appears that the facility of promoting bactericidal properties of the peritoneal fluid corresponds roughly to the growth-promoting quality of the respective proteins.

The changes observed, while reflecting accurately the relative activities of proteins in the diets used, may not be due solely to the protein levels or quality *per se*. The ration used contained neither niacin nor folic acid. It is known that the effectiveness of the mechanisms of the rat for formation of niacin is dependent upon the amount and type of protein present, and particularly on the supply of tryptophan. On the low protein rations, and particularly upon maize protein, the animals may thus have been partially defi-

cient in niacin. Fragmentary data indicate that the ability of the animal to dispense with folic acid in the diet is diminished when low levels of protein are fed. In view of previous work by K. Guggenheimer and E. Buechler (*J. Immunology* **54,** *349 (1946)*) which shows that diminished bactericidal power of the peritoneal fluid also resulted from a deficiency of thiamine, riboflavin, or vitamin A, it is possible that the relative values of the various proteins, and the amounts required for maximum activity might be altered if fed in a more complete ration.

RAPID GROWTH AND ELEVATED CELL CONCENTRATION OF AMINO ACIDS

Observations on the mode of transfer of amino acids from the maternal to the fetal organism have been limited until recently to determinations of amino acids in the maternal and fetal blood. Thirty-two years ago, A. Morse (*Bull. Johns Hopkins Hosp.* **28,** *199 (1917)*) observed that the amino nitrogen content of the plasma of the human umbilical cord as determined by the gasometric nitrous acid method was higher than in the maternal plasma. Some investigations have questioned the validity of this difference; furthermore, the more specific manometric ninhydrin method for the determination of amino nitrogen gave values for amino nitrogen in the plasma of pregnant women only about one-half those found by Morse and other research workers.

H. N. Christensen and J. A. Streicher (*J. Biol. Chem.* **175,** *95 (1948)*) have reinvestigated this problem in considerable detail as one phase of a study on the association between rapid growth and elevated concentrations of amino acids. Guinea pigs and rabbits in late pregnancy were the subjects. After the animal was fasted for twelve hours, the abdomen was opened under ether anesthesia; fetal blood was collected from the umbilical veins and maternal blood by cardiac puncture. The animals were then killed; samples of fetal and maternal skeletal and cardiac muscle were excised and analyzed for water, chloride, glutamine, glycine, and nonglutamine alpha-amino nitrogen. The amino acid concentrations of the cellular water and the distribution ratios of cellular to extracellular fluid were determined. Human blood samples were collected at normal deliveries and at elective cesarean sections from the antecubital vein of the mother and from the umbilical vein of the infant at approximately the same time.

In guinea pigs after twelve hours fasting, the glycine nitrogen content of the maternal plasma was 0.47 mg. per cent in contrast to a level of 2.40 mg. per cent for fetal plasma. Similarly, there was a much higher level of nonglycine, nonglutamine ("residual") alpha-amino nitrogen in the fetal than in the maternal plasma. Analyses of the skeletal muscle indicated that there was a much higher level of glycine and "residual" alpha-amino nitrogen in the fetus than in the pregnant female. Both the glycine and "residual" amino nitrogen concentrations of the plasma of pregnant guinea pigs were found to be considerably lower than in the plasma of nonpregnant females. It was speculated that these diminutions resulted from the avid removal of amino acids by the placenta. There was no evidence that the amino acids in the fetal plasma were in a form different from those in the maternal plasma.

In fasting rabbits, there was no appreciable difference between the glycine alpha-amino nitrogen content of the fetal and maternal plasma. However, the amount of "residual" alpha-amino nitrogen was nearly twice as high in fetal as in maternal plasma. Glycine was slightly higher and "residual" alpha-amino acid nitrogen was considerably higher in fetal skeletal muscle than in that of the mother. Both the glycine and "residual" alpha-amino nitrogen concentrations

were considerably higher in fetal cardiac muscle than in maternal cardiac muscle.

In 4 normal human deliveries and 3 cesarean sections, the glycine and "residual" amino nitrogen concentrations in fetal plasma were considerably higher than in the plasma of the mothers. The plasma of pregnant women has been shown to have a considerably lower average alpha-amino nitrogen concentration than the plasma of nonpregnant women (R. W. Bonsnes, *J. Biol. Chem.* **168,** *345 (1947)*).

A series of experiments was conducted with guinea pigs to determine the effect of feeding individual amino acids during a period of fasting before samples of blood and tissue were collected. When glycine or l-histidine or dl-methionine or l-proline were fed, high maternal plasma concentrations of these amino acids were attained, and there was a great reduction in the difference between the glycine contents of fetal and maternal plasma. Thus, the ratio of glycine nitrogen in the fetal plasma to that in the maternal plasma was reduced even as far as 1.0. In contrast, when l-glutamic acid was fed, a unique situation arose in that the ratio of glycine nitrogen in fetal plasma to that in maternal plasma was not reduced and in addition, the "residual" alpha-amino nitrogen content of the fetal plasma was increased to 37.9 mg. per cent and of the maternal plasma to 7.3 per cent.

These studies are of interest for their demonstration of the ability of the placenta to concentrate amino acids and also for the possible relation between elevated cellular contents of amino acids and rapid growth. The concentration of amino acids by the placenta presents an interesting difference from concentration by cells within their cell membranes in that two extracellular fluids are involved, separated by fetal cells, only one layer thick in these rodents, instead of one intracellular and one extracellular fluid. The high alpha-amino nitrogen concentration in the fetal plasma of guinea pigs, rabbits, and man strongly suggests that protein synthesis and growth are a result of the increased amino acid concentrations or possibly are stimulated by these marked increases in available amino acids in the circulatory system and within the cell boundaries.

Studies on the association between rapid growth and elevated amino acid concentrations were continued by H. N. Christensen, J. T. Rothwell, R. A. Sears, and J. A. Streicher (*J. Biol. Chem.* **175,** *101 (1948)*) in regenerating liver after partial hepatectomy. After removal of two thirds of the liver of the rat, the remaining lobes grow rapidly enough to double their weight within the second and third days (G. M. Higgins and R. M. Anderson, *Arch. Path.* **12,** *186 (1931)*). Adult rats were anesthetized and the median and left lateral lobes removed. For twenty hours after partial hepatectomy, the amino acid concentrations of the remaining portion of the liver did not increase any more than for controls which were subjected to laparotomy. This phase corresponded to the latent period immediately following hepatectomy described by A. M. Brues and B. B. Marble (*J. Exp. Med.* **65,** *15 (1937)*) where no great activity occurred until after the first twenty-four hours. In the next period, there was a sudden rise of mitoses and cell multiplication to a maximum followed by a rapid decline. Between the twentieth and thirtieth hours, a rise of around 50 per cent above the control levels occurred in the concentration of "residual" alpha-amino nitrogen. This increased value continued for about ninety hours. Coincident with this increase, there was a proportionately smaller increase in the "residual" alpha-amino nitrogen level of the plasma, a rise in liver glutathione, as indicated both by bound glycine and by the alpha-amino nitrogen precipitated by lead acetate, and a decrease in liver glutamine. Peptides other than glutathione did not appear to be increased in the regenerating liver lobes. There was a small but statistically significant increase in "residual" amino nitrogen in the laparotomized control rats and

an increase in the distribution ratio between liver and plasma.

It seems highly unlikely that this accumulation of amino acids could have been due solely to a reduced deaminating ability. During the latent period after partial hepatectomy, no greater amino acid concentrations had occurred in the liver and in the plasma of these rats than in the laparotomized controls. When amino acid concentrations did rise in the liver and in the plasma, the increases in the liver were far in excess of those in the plasma. It has been demonstrated that greatly accelerated deamination can be carried on in the liver without any increase in the amino acid concentrations (H. N. Christensen, J. A. Streicher, and R. L. Elbinger, *J. Biol. Chem.* **172,** *515 (1948)*). Likewise a reduced deaminating ability would not explain the coincident increase in glutathione and decrease in glutamine.

These investigations demonstrate that there is a definite association between rapid growth in fetal tissues and in regenerating liver and elevated concentrations of amino acids which are the basic components in the formation of new tissue proteins. Knowledge of the factors which are responsible for the initiation and continuation of growth responses in a single tissue or in an entire animal is extremely meager. On the basis of present knowledge, it is impossible to determine whether increased protein synthesis is initiated or promoted by the increased concentrations of amino acids and to evaluate what mechanisms might be responsible for the maintenance of these high levels of amino acids in cells and in plasma despite their rapid utilization for synthesis of tissue proteins. One problem of practical importance to be answered by the procedures used in these investigations would be the effect of the type and quality of diet upon the elevation of amino acids. Possibly an additional test of the adequacy of a diet might be its ability to permit a normal elevation of tissue amino acids in time of such stresses as pregnancy and surgery.

ASSAY OF THE GUINEA PIG STIFFNESS OR CALCIFICATION FACTOR

A. M. Bahrs and R. Wulzen (*Proc. Soc. Exp. Biol. Med.* **33,** *528 (1936)*) described in 1936 a syndrome in guinea pigs which resulted in joint stiffness and peculiar deposits of calcium salts in these animals. Since then the factor has been investigated at length by several different groups of workers. The results of W. J. van Wagtendonk and co-workers, who reported having isolated a crystalline fraction which showed high curative activity, have been discussed in this journal (*Nutrition Reviews* **1,** *292 (1943)*; **5,** *76 (1947)*). Subsequently, J. J. Oleson and co-workers (*Ibid.* **6,** *106 (1948)*) at the Lederle laboratories confirmed the existence of the stiffness and calcification syndrome and reported that a number of sterols, the most active of which was ergostanyl acetate, cured and prevented the syndrome. Their results showed that the assay was completely unsatisfactory from a quantitative viewpoint, so that it was not surprising that their figures for the activity of natural supplements such as cane juice failed by a wide margin to check those previously reported by van Wagtendonk *et al.* Similarly, the most active sterol reported was far less active than van Wagtendonk reported for his crystalline fraction. The inadequacy of the assay for obtaining quantitative results has been further emphasized in a paper by B. E. Christensen, M. B. Naff, V. H. Cheldelin, and R. Wulzen (*J. Biol. Chem.* **175,** *275 (1948)*).

In view of these results, a study of this factor by H. G. Petering, L. Stubberfield, and R. A. Delor (*Arch. Biochem.* **18,** *487 (1948)*) is of interest. These workers, too, were successful in producing the stiffness

syndrome, both on a skim milk diet and on a semipurified ration. Since mortality was high on the skim milk ration, the purified diet that contains casein (Labco), corn starch, brewers' yeast, salts, cottonseed oil, ascorbic acid, and vitamins A, D, and E was preferred for assay.

Like other observers, these workers found that many animals that were supplied commercially were suffering from wrist stiffness when first obtained. The response of such animals to active supplements was not uniform, hence they were carefully excluded from the animals to be used for assay. Animals free from such stiffness could be kept on a stock ration of oatmeal, salt, and cabbage indefinitely without developing the syndrome. After four weeks on this ration, they were transferred to the basal ration, on which, during the ensuing ten weeks, the stiffness gradually developed. The flexibility of the wrists was measured by van Wagtendonk's method. Complete absence of stiffness (flexing through 90°) was given a rating of 4; a 3° stiffness was assigned to those animals whose wrists could be flexed through 60°; a 1° stiffness was complete absence of flexing. Reliable assays could not be obtained with animals showing more than a 3° stiffness; hence these animals too were never used for assays. This degree of stiffness was secured in four to six weeks on the basal ration. At this time, assays were made by a curative technic. No spontaneous cures were found with such animals when left on the basal ration alone.

With such rigorously selected animals for assay, the authors feel that the results have considerable quantitative significance. Under these conditions, they then compared the effects of ergostanyl acetate (found curative by the Lederle group) and alpha-ergostenyl acetate. Both compounds were curative of the wrist stiffness, with indications that the latter compound was the more active of the two. In contrast to the 5 micrograms per day indicated as active by Oleson *et al.*, these authors found approximately 100 micrograms per day necessary under their conditions. It will be recalled that even the 5 microgram dose reported as active by the Lederle group was much higher than the extremely small amounts reported as active by van Wagtendonk.

Further work will be required to determine whether the modifications reported in the assay by the present group of workers suffice to give results of a satisfactorily quantitative nature. In any case, it can now be stated that the guinea pig stiffness-calcification syndrome is a definite entity, which has been produced in several laboratories, that it can be prevented, and in its milder stages cured, by dietary means, and that certain sterols appear to serve as curative agents. The fact that quantitative assay is difficult or even impossible at the present time does not change the intrinsic importance of these facts, but simply serves to emphasize the need for further work on the subject.

SKELETAL EFFECTS OF ESTROGENIC HORMONE IN SCORBUTIC GUINEA PIGS

In the growing animal, administration of excess amounts of estrogenic hormones results in a hyalinization of connective tissue, vascular walls, and cartilage and is associated with increased ossification. This response varies with species, strain, and sex of the experimental animals and the dose administered (M. Silberberg and R. Silberberg, *Arch. Path.* **36,** *512 (1943)*; W. U. Gardner and C. A. Pfeiffer, *Physiol. Rev.* **23,** *139 (1943)*). Since certain of the skeletal changes resulting from administration of estrogenic hormones were partially attributable to changes in connective tissue, M. Silberberg and R. Silberberg (*Am. J. Path.* **24,** *1019 (1948)*) studied the ways in which

the estrogenic effects upon the skeletal system were changed by alterations in the interstitial substances induced in young guinea pigs by ascorbic acid deficiency. Two groups of guinea pigs were maintained on a diet containing ascorbic acid; an additional two groups were given the same diet without the source of ascorbic acid. The animals in one of the normal and in one of the scorbutic groups were given injections of alpha-estradiol benzoate. At two, three, four, and five weeks after the beginning of the experiment, representatives of each group were killed. At autopsy, the tibias, femurs, several lower ribs, vertebrae, and whole heads were removed. The length of the tibia was measured with calipers. The bones were fixed in 10 per cent formalin and all except the heads decalcified in 5 per cent nitric acid.

The normal guinea pigs into which 0.17 mg. of alpha-estradiol benzoate in sesame oil had been injected three times weekly demonstrated the usual findings associated with administration of estrogenic hormones. The bones were thicker and harder than those in normal animals without injections. The tibias of the guinea pigs receiving estrogen injections did not grow as long as those of the normal animals. The growth zones of the epiphyses were narrowed with an increase in the size and number of cartilage cells and an increase in hyalinization and calcification of the matrix. In the diaphyses, the capillaries were inconspicuous, poorly filled, and accompanied by an increase in fibrous tissue. The trabeculae were thicker than usual due chiefly to the presence of large cartilage cells still surrounded by cartilaginous matrix. The amount of bone present was increased about 25 per cent. After four and five weeks of these injections, there was some recession in the severity of the effects induced, resulting in an increased resorption of bone, engorgement of the blood vessels, and an increase in osteoclasts.

The changes which occurred in the scorbutic guinea pigs with no estrogen injections were typical of ascorbic acid deficiency. Periosteal hemorrhages appeared in the long bones and joints after two weeks and became more extensive with the increasing duration of the experiment. After three weeks, large bead-like swellings were present at the chondro-osseous junctions of the ribs. The bones were extremely thin and fragile. There had been no increase in length of the tibias throughout the experiment. The cartilaginous ground substance in the epiphyses was loose and swollen. The epiphyseal plate became irregular in pattern due to the swelling of the matrix. Instead of the usual calcium deposits in the distal layer of the epiphyses, there appeared cartilaginous patches of deeply eosinophilic material. Capillaries of the bone marrow invaded the softened epiphyseal cartilage but without any accompanying calcification or ossification. The capillaries in the shaft were thin-walled and dilated while the trabeculae were long but thinner than usual. The bone marrow in some places was converted into a loose fibrous tissue with almost complete disappearance of trabeculae.

The changes in the guinea pigs which were scorbutic and received injections of alpha-estradiol benzoate were not entirely typical of ascorbic acid deficiency nor did they resemble completely the changes in normal animals produced by the injection of estrogenic hormones. The bones were thicker than normal but had not increased in length appreciably. Rosaries were absent except for slight swelling in one of the 8 guinea pigs. Hemorrhages were not seen in the periosteal tissue before the end of the fourth week, and then were less extensive. The growth of cartilage associated with estrogen administration to the normal animal was not altered by the scorbutic condition. However, the calcification and hyalinization of the ground substance were almost completely prevented. Although the bones were much less fragile than in the scorbutic guinea pigs without estrogen injections, excessive amounts of

bone were not formed. The investigators postulated that this was due to the inability of the intercellular matrix to be converted into bone in the absence of ascorbic acid. The thickening of the walls of blood vessels usually present after injection of estrogens into normal animals did not occur in the scorbutic guinea pig. The delay in the onset of scorbutic manifestations was demonstrated in the histologic preparations by the maintenance for almost the full experimental period of regular epiphyseal plates and thin but regular and numerous trabeculae. An interesting observation which paralleled those in normal animals was the reduced effect of the estrogenic hormones in the latter part of the experiment. This reduced effectiveness was manifest by an increase in the severity of scurvy and was attributed to an adaptive ability of the guinea pig to accommodate itself to these large doses of alpha-estradiol benzoate.

These investigations are of interest in that they elucidate a little further the effect of the estrogens on the skeletal system and the dependence of this reaction on the existence of a normal condition in the ground substance to be calcified. Of greater interest, the unexpected observation that administration of alpha-estradiol benzoate prolonged the survival of scorbutic guinea pigs and reduced the severity of all the classic signs of scurvy, raises many questions about the interrelations of estrogens and ascorbic acid.

NIACIN DEFICIENCY ANEMIA IN SWINE

Anemia has long been associated with pellagra, but there has been no proof that it results from niacin deficiency. In dogs, however, P. Handler and W. P. Featherston (*J. Biol. Chem.* **151,** *395* (*1943*)) were able to show that when animals suffering from acute blacktongue were given physiologic saline parenterally to relieve the severe dehydration and hemoconcentration, a severe normocytic or macrocytic anemia appeared which responded rapidly and specifically to administration of synthetic niacin, but not to purified liver extracts. Using a different technic of repeatedly producing niacin deficiency in dogs until niacin alone fails to benefit the animal, W. R. Ruegamer, N. Torbet, and C. A. Elvehjem (*Federation Proceedings* **6,** *287* (*1947*)) were able to show that under these conditions both folic acid (pteroylglutamic acid) and refined liver extract were also necessary to permit growth and relieve anemia in their animals.

To study further the role of niacin in anemia, G. E. Cartwright, B. Tatting, and M. M. Wintrobe (*Arch. Biochem.* **19,** *109* (*1948*)) have conducted experiments on niacin deficient swine. Twenty weanling pigs, 21 to 28 days of age, were divided into a control group of 8 animals, which received niacin in addition to the basal ration, and a group of 12 pigs which received the basal ration only. The basal rations were semi-synthetic in character, contained no added niacin, but were low in protein (10 per cent casein) to prevent niacin synthesis from excess tryptophan. On these rations, anemia developed to significant proportions within fifty days, with maximum development between sixty and one hundred and twenty days. At this time, deficient animals showed hemoglobin levels ranging from 5.9 to 9.0 g. per 100 ml., and a hematocrit value of 18.8 to 28 ml. per 100 ml. of blood. Corresponding ranges in the control animals were 11.1 to 13.8 g. for hemoglobin, and 35 to 40 ml. for the hematocrit. With only one exception, the anemia was normocytic and normochromic. Intramuscular injection of niacin in such animals produced almost immediate reticulocytosis and a gradual rise over a sixty day period in the hematocrit value (from 21 to 38 ml. per 100 ml. of blood). An increase in the level of

protein from 10 per cent to 26 per cent of the ration produced a similar effect, which was further increased by additional niacin. Refined liver extract was ineffective in curing this anemia.

Five of the animals were given small doses (25 to 100 mg.) of niacin at intervals throughout a two hundred and sixteen day period. Such doses temporarily delayed progress of the anemia, and produced a significant but unsustained rise in the hematocrit value. Finally, refined liver extract was administered, but failed to show a curative effect. Subsequent administration of niacin was effective.

Thus niacin is necessary for normal erythropoiesis in the pig, a specific type of anemia developing in its absence which is prevented only by administration of niacin or of sufficient protein to provide niacin by synthesis. The authors mention that a marked ataxia also developed in a number of the deficient animals, but do not consider this condition further in the present report.

DETERMINATION OF PANTOTHENIC ACID IN FOODS

Long before the characterization of pantothenic acid, this vitamin was known to be present in tissues in a "bound" form which had no growth-promoting activity for yeast or lactic acid bacteria (*see* R. J. Williams, *Advances in Enzymology* **III,** *253* (*1943*)), and autolysis of fresh tissues was early observed to liberate additional quantities of the vitamin in a form utilizable by microorganisms. Subsequently, V. H. Cheldelin, M. A. Eppright, E. E. Snell, and B. M. Guirard (*Univ. Texas Pub.* **4237,** *15* (*1942*)) noted that in many tissues the amounts of pantothenic acid liberated in a microbiologically available form could be materially increased over those obtained by autolysis alone by the combined digestive action of papain and "clarase." Subsequently, M. Ives and F. M. Strong (*Arch. Biochem.* **9,** *251* (*1946*)) studied several alternative digestive procedures and recommended use of mylase P (a fungal enzyme of high saccharolytic and phosphorolytic activity similar in action to clarase or takadiastase) for liberation of the vitamin preparatory to microbiologic assay. Most of the more recently published investigations dealing with the pantothenic acid content of foodstuffs have utilized these or similar digestive procedures.

The few comparative assays which are available have always shown that chick-growth assays of certain materials yield higher values for their pantothenic acid content that do microbiologic assays (D. E. Pennington *et al.*, *Univ. Texas Pub.* **4137,** *14* (*1941*); T. H. Jukes, *Biol. Symposia* **XII,** *263* (*1947*)).

A probable reason for this discrepancy became evident following the discovery that pantothenic acid was a component of a widely distributed coenzyme essential for certain acetylations in the body (*Nutrition Reviews* **5,** *331* (*1947*)), that the pantothenic acid present in this "coenzyme A" was not available for growth of microorganisms used for assay, and that it was not liberated in available form by digestive procedures previously used for microbiologic assay (F. Lipmann *et al.*, *J. Biol. Chem.* **167,** *869* (*1947*)).

The liberation of pantothenic acid from tissues has now been reinvestigated by J. B. Neilands and F. M. Strong (*Arch. Biochem.* **19,** *287* (*1948*)). These authors cite unpublished data of G. D. Novelli and F. Lipmann which show that the pantothenic acid can be liberated from coenzyme A in microbiologically available form by the combined action of an unidentified enzyme from pigeon liver and intestinal phosphatase. A more practicable source of the former enzyme was found to be chicken liver, and a method for preparation of concentrates of it is described.

A procedure was evolved for treatment of natural samples with both enzymes suitable for general application to samples.

As an illustration of the action of the two enzymes, a boiled extract of chicken liver contained only 0.38 microgram of pantothenate per milliliter by direct assay. Following enzymatic treatment, the assay value was 36.8 micrograms per milliliter. The authors next compared the values obtained for the pantothenic acid content of several natural materials following (a) no treatment, (b) digestion with mylase P, and (c) digestion with liver enzyme plus intestinal phosphatase. Illustrative figures (micrograms per gram) following these three treatments were: spinach, 7.2, 11.5, and 30; whole liver powder, 50.4, 70, 400; fresh egg, 9.9, 13, 53. In general the improved procedure showed much higher pantothenic acid contents for these materials than had been previously observed by microbiologic assay.

It thus appears that most of the values so far recorded in the literature for the pantothenic acid content of natural materials are in error, and are low. The study illustrates the need for more extensive checking of microbiologic assays against animal assays, to insure that unavailable forms are not escaping detection. Especially is this true for the more recently discovered vitamins, such as pantothenic acid, folic acid, vitamin B_6 (pyridoxal, pyridoxamine, and pyridoxine), and biotin all of which exist in tissues in firmly bound forms, and which are rarely determined other than by microbiologic methods. The premature standardization of vitamin assay methods, including extraction procedures, would also appear dangerous, since it is only by the constant checking of analytical values obtained by one method against those obtained by another that the existence of the various bound forms in which many vitamins appear to occur can be detected.

CHEMICAL CHANGES ASSOCIATED WITH VARIOUS EXPERIMENTALLY INDUCED DEFICIENCIES IN SWINE

Hitherto, comparatively little information has been available concerning the plasma iron, plasma copper, and free erythrocyte protoporphyrin in experimental animals. The free erythrocyte protoporphyrin represents a minute fraction of the protoporphyrin in the red cell and is that part which has not been incorporated in hemoglobin.

Data for normal swine were presented by G. E. Cartwright and M. M. Wintrobe (*J. Biol. Chem.* **172,** *557 (1948)*). In that communication it was also reported that in pyridoxine deficient swine the values for plasma copper are reduced somewhat below normal, the plasma iron is very much greater than normal, and free erythrocyte protoporphyrin is markedly reduced. Because of the small amount of free protoporphyrin in the erythrocytes of the pyridoxine deficient animals, and because a fall in this value occurs long before the development of anemia, it was suggested that the fundamental disturbance in anemia due to this deficiency may be a failure in protoporphyrin synthesis.

In a later report (*J. Biol. Chem.* **176,** *571 (1948)*) the same investigators present the results of similar determinations in swine with anemia due to protein deficiency, iron deficiency, and acute hemorrhage. In addition, values for the iron-binding capacity of the serum in normal, protein deficient, and iron deficient swine are given.

In protein deficient animals, the anemia was usually mild in degree. It was normocytic and normochromic in type and not accompanied by reticulocytosis. There was no reduction in the number of leucocytes or blood platelets. The chemical examination of the blood, while revealing hypoproteinemia, especially hypoalbuminemia, showed no significant alteration in free erythrocyte protoporphyrin from the normal

but there was a significant lowering in the plasma iron. Along with the reduction in total serum proteins, there was a proportional reduction in the iron-binding capacity of the serum. The iron-binding protein of the plasma has been shown to be a $beta_1$-globulin (fraction IV_7 of Cohn) but the decrease in iron-binding capacity was correlated with the lowering in albumin rather than globulin. This may be explained by the fact that the iron-binding protein is a globulin of low molecular weight (90,000). Low plasma copper values were also observed in the hypoproteinemic animals and the degree of reduction was found to be correlated closely with the degree of hypoalbuminemia.

As would be expected, the anemia in the iron deficient pigs was severe in degree and microcytic and hypochromic in type and was accompanied by some increase in reticulocytes. There was no significant alteration in the free erythrocyte protoporphyrin or plasma copper but a pronounced hypoferremia developed. Determinations of the iron-binding capacity of the serum were made, unfortunately, in only 3 iron deficient pigs. In 2 of these, the iron-binding capacity was reduced and, in one, no change from normal was noted. This contrasts with findings in human subjects with iron deficiency, in whom an increase in iron-binding capacity is found, even though the plasma iron is very low.

These studies are of interest from several points of view. Because protoporphyrin is believed to be synthesized from certain amino acid precursors, it is significant that a normal amount of free protoporphyrin in the erythrocytes was observed in hypoproteinemic animals. This is in accord with the conclusion of Whipple and his group (F. S. Robscheit-Robbins *et al.*, *J. Exp. Med.* **72,** *479 (1940)*) who pointed out that the body gives preference to hemoglobin production as compared with serum protein production. Not only did the hypoproteinemic animals maintain a normal free erythrocyte protoporphyrin, but they were capable of responding with a substantial rise in this fraction following severe hemorrhage and infection, respectively.

The amount of iron carried in the serum appears to be limited, at least under normal conditions, by the amount of iron-binding protein in the blood. In the protein deficient animals, this substance appeared to be markedly reduced, although the saturation of the protein with iron was not significantly higher in the deficient animals as compared with the controls.

The moderate decrease in plasma copper observed in the animals deficient in protein, as well as its correlation with the degree of hypoalbuminemia, uggest that copper in the plasma is also bound by a protein and that in the deficient animals this protein was markedly reduced.

The question has been raised (G. R. Greenberg *et al.*, *J. Clin. Invest.* **26,** *121 (1947)*) whether a disturbance in protein metabolism may explain the chemical changes which are observed in association with the anemia of infection. The fact that in protein deficiency there was no abnormality in erythrocyte protoporphyrin, only a slight reduction in plasma iron, and a significant reduction in plasma copper contrasts with the finding in the anemia of infection of increased protoporphyrin, hypoferremia, and hypercupremia. Thus, it would seem that the changes observed in the anemia of infection are not due primarily to a deficiency of protein.

In human subjects, microcytic hypochromic anemia due to a deficiency of iron is accompanied by a marked increase in erythrocyte protoporphyrin and plasma copper. The normal erythrocyte protoporphyrin and plasma copper values in the iron deficient pigs are, therefore, puzzling. One possible explanation is that there is a species difference since the normal values in these two species are not alike. Another possibility is that the high erythrocyte proto-

porphyrin values and hypercupremia which accompany such anemia in man are not due to iron deficiency *per se* but are due to some other cause. The point is worthy of further study.

In another report G. E. Cartwright, E. L. Smith, D. M. Brown, and M. M. Wintrobe (*J. Biol. Chem.* **176,** *585 (1948)*) describe the results of electrophoretic analyses of serums of normal and hypoproteinemic swine. They confirm the observations of previous workers that a prolonged dietary restriction of protein results in a marked diminution in both the relative and absolute amount of albumin, while a relative increase occurs in globulin, especially alpha-globulin. Of interest is the fact that the degree of hypoproteinemia in these animals was much greater as determined by electrophoresis than by the chemical method. Also of interest is the fact that, although there was a slight reduction in the beta-globulin fraction as measured electrophoretically, the metal-binding protein as determined by the total iron-binding capacity was reduced about 65 per cent.

URINARY EXCRETION OF AMINO ACIDS IN LIVER DISEASE

Each of the amino acids varies greatly in the percentage of ingested acid which is excreted. In general, the amount excreted is dependent on the amount ingested (*Nutrition Reviews* **5,** *213 (1947)*). The presence of amino acids in the blood and their near absence from the urine is suggestive that renal retention of these compounds is usually efficient. Although the renal reabsorption of the various amino acids has been studied extensively, the exact mechanisms involved are not completely known (*Ibid.* **5,** *43 (1947)*).

It is fairly well established that in man excessive excretion of nitrogen in the urine follows trauma and surgical operations (*Nutrition Reviews* **5,** *329 (1947)*). Excessive excretion of specific amino acids in the urine also has been reported. Histidine metabolism is reported to be disturbed in preeclamptic toxemia and, in fact, histidinuria is reduced considerably, or may be completely absent, in the case of a patient who has symptoms of severe preeclamptic toxemia (*Ibid.* **1,** *134 (1943)*).

Thirty-seven years ago, S. A. K. Wilson (*Brain* **34,** *295 (1912)*) called attention to a specific cerebral degeneration often associated with cirrhosis of the liver. The disease is known as "hepatolenticular degeneration," or, commonly, as "Wilson's disease." Despite many investigations of this condition it has never been determined whether the brain is injured by some substance produced by the liver or whether the brain and liver suffer together from some unknown agent. Hepatolenticular degeneration is a familial condition which affects nearly all siblings in one generation, usually develops in adolescence, and is, as a rule, progressive. In some cases cirrhosis of the liver may be advanced and may be present many years before cerebral symptoms appear. It is stated that, in some cases, the patient may die of cirrhosis without obvious neurologic changes ever developing. In most instances, however, the cirrhosis has given rise to few symptoms other than dyspeptic ones. Apparently the nervous manifestations present vary greatly from family to family. The classic patient is one who suffers severe immobilization and disability and distortion of posture, with or without tremor. Another variety is characterized by an unusual tremor which may be the only symptom present. In this latter group the characteristic pigmentation of the cornea (Kayser-Fleischer ring) is always present; this pigmentation has not been found in any other neurologic condition.

In a report of L. Uzman and D. Denny-Brown (*Am. J. Med. Sci.* **215,** *599 (1948)*), hepatolenticular degeneration has been fully

discussed and described, including variations and important points in diagnosis. Because of the recently accumulated data which show that deficiency of certain amino acids in animals may lead to damage of the liver, these authors felt that it might be of interest to inquire into the relation of amino acid metabolism to cirrhosis in hepatolenticular degeneration. It is well known, of course, that severe damage to the liver cells will seriously disturb amino acid metabolism and, for this reason, it was obvious that the only suitable cases to study would be those in which hepatic cirrhosis had not yet developed or those in which a minimum amount of hepatic damage was present.

The patient studied by these investigators had a minimum amount of liver damage and the history, physical findings, and laboratory studies are given in detail. The studies on amino acids were carried out on twenty-four hour specimens of urine and aliquots were taken for determination of amino nitrogen by the Soerensen formol titration, amino nitrogen determination by the Van Slyke ninhydrin method, and small portions for separation and identification of alanine, glutamic acid, and asparaginic acid.

Abnormally high concentrations of amino nitrogen were found in the urine, the amounts varying from 1500 to 1800 mg. per twenty-four hours (normal 100 to 200 mg. daily). The patient at this time was on a high protein diet. When the patient was given a diet low in protein (15 to 20 g. daily) there was only slight reduction of the amount of amino nitrogen excreted in the urine. During this study the nonprotein nitrogen in the blood varied from 32 to 36 mg. per 100 ml. and the blood urea remained about 22 mg. per 100 ml. When it was established that the patient was excreting large amounts of amino nitrogen, further studies of the urine were made by use of the paper partition chromatography method, as simplified by C. E. Dent (*Lancet* **II,** *637 (1946)*). From these studies it was found that ten to twelve different amino acids were present in the urine and that a specific amino acid was not present in excess of the others. Thus, apparently this individual did not excrete any preponderance of one amino acid in the urine.

In cysteinuria, alkaptonuria, and phenylketonuria it is generally accepted that defects in certain of the metabolic systems account for these peculiar syndromes. The data presented by these authors certainly suggest that hepatolenticular degeneration presents a fundamental defect in the metabolism of amino acids.

This type of study is stimulating and with increased refinement in measurement of specific amino acids and increased knowledge of their selective reabsorption by the kidney, undoubtedly many frontiers will be opened to the scientist interested in clinical medicine and its nutritional problems.

BIOSYNTHESIS OF PURINES AND PYRIMIDINES

Purines and pyrimidines are essential substances in all cells. They are components of nucleic acids; the purine adenine also occurs in essential nucleotides such as flavin and pyridine nucleotides, and a pyrimidine occurs in the thiamine molecule. The animal body does not depend on preformed purines or pyrimidines in the diet, these apparently being synthesized from ammonia and readily available carbon sources. Little is known of the intermediate steps in the synthesis of purines and pyrimidines, although several studies have indicated that folic acid is implicated in the synthesis of the pyrimidine thymine, and vitamin B_{12} in the synthesis of thymidine, the nucleoside of thymine (*Nutrition Reviews* **7,** *19, 49 (1949)*). J. L. Stokes (*J. Bact.* **48,** *201 (1944)*) suggested that folic acid may function as a coenzyme in a system responsible for the

synthesis of thymine or a thymine-like compound, which in turn is used for the synthesis of nucleic acid.

Analytical methods for nucleic acids have been sufficiently well developed that the effect of folic acid on nucleic acid synthesis might be tested directly. W. H. Prusoff, L. J. Teply, and C. G. King (*J. Biol. Chem.* **176,** *1309 (1948)*) have been led in this direction by measuring nucleic acid synthesis in Lactobacillus casei. Both ribonucleic acid and desoxyribonucleic acid were measured in these bacteria when grown in normal and pteroylglutamic acid deficient mediums. The difference in function of these two nucleic acids is not known, but their distribution in the nucleus and protoplasm of the cell is very different. In the pteroylglutamic acid deficient cells there was a marked decrease in desoxyribonucleic acid, although a normal content of ribonucleic acid was found. In accord with previous observations on the role of pteroylglutamate in the synthesis of thymine, the addition of thymine to the pteroylglutamate deficient mediums resulted in a correction of the decreased desoxyribonucleic acid synthesis. Under these conditions, in fact, the content of both nucleic acids was increased over that in the cells grown in the control mediums. As a test of the specificity of pteroylglutamate on nucleic acid synthesis, the organism was grown in mediums deficient in riboflavin or biotin. Under these conditions both the ribonucleic and desoxyribonucleic acid content of the cells were somewhat increased, hence the nucleic acid change in pteroylglutamate deficiency was not simply related to decreased growth of the cells. These studies not only confirm the role of pteroylglutamate in the synthesis of pyrimidine, but at the same time bring us closer to a direct relation of a vitamin with a long recognized component of cellular structure and metabolism.

The new vitamin B_{12} also appears to be concerned in the synthesis of purine or pyrimidine components of the cell. W. Shive, J. M. Ravel, and W. M. Harding (*J. Biol. Chem.* **176,** *991 (1948)*) have advanced this relation by studying the requirements of Lactobacillus lactis Dorner, the test organism for vitamin B_{12} assay. They found that in the absence of vitamin B_{12}, both purines and thymidine are essential for growth. In these experiments a constant supplement of purines (guanylic acid, guanine plus hypoxanthine, or adenine plus guanine) was used with a variable supplement of thymidine, or conversely a constant supplement of thymidine with variable additions of purines. In the presence of the purines, thymidine increased growth; in the presence of thymidine, purines increased growth. The purine requirement was not specific—guanylic acid was the most active of the single purines, but combinations of adenine plus guanine or hypoxanthine plus guanine were nearly as efficient. On the other hand, thymidine appeared specific; thymine could not replace thymidine. Three different liver concentrates of vitamin B_{12} produced growth of the organism, replacing the combined requirement of purines and thymidine. The presence of purines in the medium did spare the vitamin B_{12} needs for growth, but not greatly. Thus it is indicated that vitamin B_{12} is effective in the synthesis of both purines and thymidine. The nonactivity of thymine, which with other organisms appears to require folic acid for synthesis, illustrates a functional difference between folic acid and vitamin B_{12}. It might appear that folic acid is required for thymine synthesis and vitamin B_{12} for thymidine synthesis, but the precise differences in function are not yet entirely clear.

ALPHA-TOCOPHEROL IN HEART DISEASE

One of the most intriguing problems in medical science has been the study of the cause, or causes, of cardiac failure. Approaches have been made from many angles but to date the exact mechanism which takes place in the cardiac muscle is not entirely known.

One approach to the problem has been the study of cardiac changes in various nutritional deficiencies. It is known that in rats fed a diet low in potassium, necrosis of the myocardial fibers results (*Nutrition Reviews* **1,** *10* (*1942*)). Areas of focal or diffuse necrosis in the myocardium of pigs that have died of thiamine deficiency also have been reported (*Ibid.* **1,** *314* (*1943*)), and the possible role of vitamins in the metabolism of cardiac muscle has been discussed (*Ibid.* **3,** *236* (*1945*)). Edema of the heart and pericardium of chicks fed a diet of skimmed milk is said to be prevented by administration of d–l-alpha-tocopherol (H. R. Bird and T. G. Culton, *Proc. Soc. Exp. Biol. Med.* **44,** *543* (*1940*)). W. M. Govier, N. Yanz, and M. E. Grelis (*J. Pharmacol. Exp. Therap.* **88,** *373* (*1946*)), in a study of homogenates of hearts of guinea pigs with avitaminosis E, concluded that vitamin E was important in metabolism of the cardiac muscle. While studying the role of vitamin E in the nutrition and reproduction of cattle, T. W. Gullickson and C. E. Calverley (*Science* **104,** *312* (*1946*)) noted that cattle fed vitamin E-free rations throughout their entire life span often died suddenly without evident cause as revealed by gross necropsy examination. In one of the animals studied, the electrocardiogram showed abnormal changes, and microscopic studies of the heart revealed changes in the Purkinje network.

These studies of nutrition in relation to the heart muscle are stimulating, but even a cursory study soon reveals that many more experimental studies will have to be carried out before definite conclusions can be drawn Despite these fragmentary data on animals, A. Vogelsang and E. V. Shute (*Nature* **157,** *772* (*1946*)) reported, while studying the effect of high dosage of alpha-tocopherol acetate on purpura in human beings, that in their series those patients with coronary heart disease were improved. These investigators administered 200 to 600 mg. of vitamin E daily for months and noted what they described as a marked effect on those patients who had congestive heart failure and angina pectoris and, in addition, noted increased tolerance to exercise and diminished anginal pain, as well as a pronounced diuretic effect. Later, A. B. Vogelsang, E. V. Shute, and W. E. Shute (*Med. Record* **160,** *279* (*1947*)) reported results obtained in 126 patients treated with vitamin E; in this report it was stated that most patients with angina pectoris had relief of pain, hypertension was decreased, and arrhythmias were regulated.

These very poorly reported clinical studies have already received decidedly unfavorable critical appraisal (*Nutrition Reviews* **5,** *164* (*1947*)). Further unfavorable appraisal of the work of Vogelsang and his associates has been reported recently in a study by S. Baer, W. I. Heine, and D. B. Gelfond (*Am. J. Med. Sci.* **215,** *542* (*1948*)). These authors treated and followed 22 patients, of whom 11 had congestive heart failure and 5 angina pectoris. The remaining 6 had hypertension or arteriosclerotic heart disease or both without failure or angina. These patients were given 400 mg. of vitamin E daily in divided doses. This dose was reduced to 300 mg. daily within two to four weeks after the study was initiated. Then the patients were followed some three to six months and electrocardiograms were made at intervals of two to four weeks. Some of the patients had already been taking such medications as digitalis, mercurials, and phenobarbital and they were allowed to continue taking these. In some cases, however, use of the medications was discontinued after vitamin E was given. This was done to

determine whether recurrence of cardiac failure would be prevented. In those patients who exhibited some symptomatic response to the administration of vitamin E, tablets of calcium gluconate, which appeared similar to the vitamin E tablets, were given as placebos. These patients were then examined critically and the tabulated data are presented in the article.

In none of the 22 patients was any marked, or even moderate, improvement noted following administration of vitamin E. Six of the 22 patients were very questionably improved and the remainder, as stated, showed no change or they became markedly worse. The authors humbly point out that their series of cases is small and that there are few objective data, such as circulation time, exercise tolerance test, venous pressure studies, and the like, but that the results were so discouraging that there seemed to be little reason to carry out a more objective experiment.

Certainly this well tabulated, critical clinical study, and others, should discourage physicians or laymen from recommending the use of vitamin E in the treatment of coronary heart disease, congestive heart failure, or hypertension. It also should discourage individuals from reporting clinical impressions without adequate controls and without adequate scientific data to support their impressions. Such a report not only is useless, but it wastes the time of the careful clinician or scientist who has to repeat the work. In the layman who may have the disease, such a report may instill false hopes for cure and in some instances might even cause him to discontinue taking useful medications for those of questionable value.

ZINC CONTENT OF HUMAN BLOOD AND ITS CONSTITUENTS

The study of zinc metabolism and of the zinc content of the various tissues in human beings or in experimental animals has been until recently dependent upon the development of adequate analytical methods. W.R. Todd and C. A. Elvehjem (*J. Biol. Chem.* **96,** *609 (1932)*) investigated the various gravimetric, turbidimetric, and colorimetric methods which were available at that time and found each of them to be deficient in one or more aspects. Diphenylthiocarbazone (dithizone) has been used for a variety of analyses of certain metals in industrial processes. Later workers adapted the dithizone reaction for the colorimetric determination of zinc and other heavy metals in plant and animal tissues.

A refinement of the dithizone method has been reported by B. L. Vallee and J. G. Gibson (*J. Biol. Chem.* **176,** *435 (1948)*) who were able to analyze with considerable accuracy samples of whole blood, plasma, erythrocytes, leucocytes, and animal tissues containing as little as 1 to 5 micrograms of zinc. Using the dithizone method, these investigators (*Ibid.* **176,** *445 (1948)*) proceeded to determine the zinc content of normal human blood, plasma, leucocytes, and erythrocytes. Thirty-one normal individuals, 15 males and 16 females, ranging in age from 18 to 45 years, were studied. In addition, blood samples from 5 hospitalized medical patients without apparent blood dyscrasias were analyzed. Blood samples were taken in the morning with the subject in fasting condition, but not otherwise in basal conditions. A series of fifteen consecutive blood samples was collected at one to four day intervals from the same normal individual, a 22 year old male. This series was studied to determine the normal variation within one individual.

The average zinc content of whole blood of the 31 normal individuals was 8.8 micrograms per milliliter with a range of 5.2 to 14.8. The average zinc content of plasma was considerably lower with an average of 3.0 micrograms per milliliter with a range of

1.2 to 11.4. Erythrocytes were found to have a much lower zinc content than the leucocytes with an average for the former of 0.00134 microgram per million cells in contrast to 0.032 microgram per million cells for the latter. The average zinc content of erythrocytes was also expressed as 14.4 micrograms per milliliter of packed cells with a range of 9.1 to 18.4. No value for the zinc content of leucocytes was expressed on the basis of milliliters of packed cells because of the difficulties in determining their small volumes accurately. No significant difference was found between the values for males and females. Averages for the 5 medical patients with no apparent blood dyscrasias were very close to the averages listed above. There was a definite variation in the values for the zinc content of whole blood and its components from day to day in the subject who was followed over a period of twenty-eight days. The extent of the variation was not as great as that observed among the 31 normal individuals, but the averages of the 15 consecutive determinations corresponded closely to the respective averages for the 31 subjects.

These investigations give an accurate method for determination of minute amounts of zinc in biologic materials and also a survey of the zinc content of normal human whole blood, plasma, erythrocytes, and leucocytes. With the possible exception of the zinc determinations on leucocytes, the methods are sufficiently sensitive as to be adaptable to studies in small experimental animals. Thus studies of the distribution of zinc in normal and deficient animals can proceed at the same time that studies are continued in human beings to determine whether the zinc content of the various blood components is altered in specific blood dyscrasias and how treatment of these disease syndromes may alter the metabolism of zinc.

A POSSIBLE CATALYTIC FUNCTION FOR INOSITOL

The nutritional significance of inositol has been summarized previously in this journal (*Nutrition Reviews* **4,** *163* (*1946*)). Like choline, it is apparently an integral component of certain lipids of living tissues. Also like choline, it may serve other functions as well.

A clue to a possible role of inositol was the observation of R. J. Williams, F. Schlenk, and M. A. Eppright (*J. Am. Chem. Soc.* **66,** *896* (*1944*)) that a highly purified preparation of pancreatic amylase contained relatively large amounts of inositol (4.1 mg. per gram). They concluded that inositol appeared to be a constituent of the enzyme. Subsequently, K. H. Meyer, E. H. Fischer, and P. Bernfeld (*Helvet. Chim. Acta* **30,** *64* (*1947*)) obtained evidence that pancreatic amylase could be dissociated to yield a heat-stable coenzyme and a labile protein; recombination, however, failed to restore enzymatic activity.

The subject has been further investigated by R. L. Lane and R. J. Williams (*Arch. Biochem.* **19,** *329* (*1948*)). Highly purified pancreatic amylase was prepared by fractionation of commercial pancreatin. Three such purified preparations contained 3.0, 3.8, and 3.9 mg. of inositol per gram (yeast assay). The purified enzyme was then incubated in solution with the gamma-isomer of hexachlorocyclohexane. This structural analogue of inositol had been previously shown to act as a competitive inhibitor of inositol function in yeast (S. Kirkwood and P. H. Phillips, *J. Biol. Chem.* **163,** *251* (*1946*)) and Nematospora gossypii (H. W. Buston, S. E. Jacobs, and A. Goldstein, *Nature* **158,** *22* (*1946*)), and it was hoped that if inositol were in fact essential for the activity of pancreatic amylase, this activity might be lost when the chlorinated analogue of inositol were added. Aliquots of the mixture of enzyme and inositol analogue were assayed for enzymatic activity at intervals and essentially complete loss of enzymatic activity was

observed over a fifteen hour period. Loss of activity proceeded slowly, presumably because of a slow replacement of inositol in the enzyme protein by the inactive analogue. Very little decrease in enzymatic activity of a control solution of the enzyme occurred during the same time interval. Increasing amounts of gamma-hexachlorocyclohexane exerted increasing inhibitory effects; inhibition was essentially complete when the molar quantity of added inhibitor was fifty times that of the inositol present in the enzyme. Simultaneous incubation of enzyme, inhibitor, and inositol showed that the enzyme was protected against inactivation by the added inositol.

These results appear to demonstrate that inositol plays a role in the activity of pancreatic amylase. It was of interest, therefore, to determine whether inositol was generally present in starch-splitting enzymes. A concentrate of the beta-amylase from "clarase" contained only about one-sixth as much inositol as did pancreatic amylase of the same activity. Also in contrast to pancreatic amylase, incubation with gamma-hexachlorocyclohexane resulted in no inhibition of this enzyme. It would appear on this basis that inositol was not an active constituent of this enzyme.

Elucidation of the physiologic role played by various vitamins is always fascinating, and is of the greatest biochemical importance. The present data certainly indicate, as the authors conclude, that inositol plays an essential role in the activity of pancreatic amylase. It would be coincidence indeed if the inactivating action of the inositol analogue, the protective action of inositol against this inactivation, and the high inositol content of the concentrated enzyme were all fortuitous circumstances. Nevertheless, the importance of the subject is such that additional evidence to strengthen the conclusions reached would be welcomed.

EFFECT OF SEVERE HEAT TREATMENT ON AMINO ACIDS IN PORK

Relatively little is known about the effect of either mild or severe cooking procedures on the amino acid composition and on the digestibility of the proteins in edible meats. Most studies where normal cooking procedures were used indicated that heating caused slight and possibly insignificant reductions in the amino acid contents of various types of meats (B. S. Schweigert, I. E. Tatman, and C. A. Elvehjem, *Arch. Biochem.* **6,** *177* (*1945*); I. T. Greenhut, R. L. Potter, and C. A. Elvehjem, *Ibid.* **15,** *459* (*1947*); O. H. M. Wilder and H. R. Kraybill, *J. Nutrition* **33,** *235* (*1947*)). When small losses were reported, the question could always be raised that the experimental circumstances might have prevented sufficiently accurate measurements to detect the cause of the loss.

J. F. Beuk, F. W. Chornock, and E. E. Rice (*J. Biol. Chem.* **175,** *291* (*1948*)) have conducted a series of investigations in which fresh and cured pork were cooked under extreme conditions to accentuate any loss of amino acids. Fifty pounds of lean pork trimmings were ground and mixed thoroughly. One-half was cured by mixing with an approved standard commercial curing formula; the other half was untreated. Both lots were kept at 2 to 3°C. for forty-eight hours until the one-half was amply cured. Both halves were then packed and sealed in cans under vacuum. Several cans of fresh and cured pork were stored at −29°C. as the raw controls. The remaining cans were autoclaved for twenty-four hours at 112°C., cooled immediately, and stored at −29°C. Acid hydrolysates of representative samples of raw fresh, raw cured, autoclaved fresh, and autoclaved cured pork were made by refluxing 10 g. portions with 100 ml. of eighth-normal hydrochloric acid for eight hours. Enzyme digests were prepared by incubating 10 g. portions in 100 ml. of water

at pH 7.5 with 100 mg. of commercial trypsin for twenty-four hours and then with 100 mg. of erepsin at pH 7.5 for an additional twenty-four hours. Digests were then brought to pH 4.5 and heated to inactivate the enzymes. Prior to analysis of aliquots for amino acids, all enzyme digests were filtered to remove particulate residues. In later parts of the investigation, certain enzyme digests were hydrolyzed with acid under the same conditions as described above. Suitable aliquots of acid hydrolysates, enzyme digests, and enzyme-acid hydrolysates were analyzed for threonine, leucine, isoleucine, valine, methionine, phenylalanine, cystine, lysine, arginine, and histidine by microbiologic procedures and for histidine by the chemical method of A. A. Albanese, J. E. Frankston, and V. Irby (*Ibid.* **160,** *441 (1945)*).

Analyses of the acid hydrolysates of the autoclaved samples indicated that approximately 100 per cent of the amino acids except cystine was retained. Only 56 per cent of cystine was found in both fresh and cured autoclaved samples of pork. Acid-labile tryptophan was analyzed for in alkaline hydrolysates and found to have been completely retained. The curing mixture apparently did not affect the quantity of amino acids present in the raw cured sample nor affect retention of amino acids during autoclaving.

Analyses of the enzyme hydrolysates were radically different from those on the acid hydrolysates and indicated that the apparent amino acid retention ranged from 27 per cent for cystine to 56 per cent for leucine in autoclaved fresh pork, and from 33 per cent for cystine to 65 per cent for isoleucine in the autoclaved cured pork. These findings were stated to be in accord with unpublished determinations of biologic values of these materials as protein sources made by the rat growth method. In the latter studies, the addition of cystine to the autoclaved proteins still did not allow a good rate of growth, but the replacement of autoclaved proteins by raw proteins permitted rapid increases in body weight.

Analyses of acid hydrolysates of the unfiltered enzyme digests indicated that the process of acid hydrolysis markedly increased the amino acid levels of the autoclaved samples but did not increase the amino acid levels of the raw samples. These increases were sufficient to indicate that there had been no actual losses of amino acids except cystine and that enzyme digestion had been inadequate to free all the amino acids in the autoclaved samples.

A part of the lower amino acid content revealed by enzymatic digestion had been due to the filtering off of insoluble protein particles prior to analyses for individual amino acids. Some 9 to 16 per cent of the total nitrogen in the enzymatic digests of autoclaved samples was in the insoluble residue and lost to the amino acid analyses. In comparison, only 3 to 5 per cent of the total nitrogen in the digests of raw samples was found in the insoluble residues. In addition, there must have been present in the filtrates a considerable amount of amino acids in such combinations as to be unavailable to the bacteria used in the assays. From parallel rat growth experiments, it appears that some similar situation must exist which makes the proteins in the autoclaved samples lower in their biologic values.

One would expect from these studies that the retention of all amino acids with the possible exception of cystine would be good in ordinary cooking procedures. At present, our knowledge of the retention of cystine during cooking needs to be supplemented considerably. The most interesting observation of this investigation would seem to be the reduced ability of autoclaved pork proteins to be digested by trypsin and erepsin. This cooking procedure was, without doubt, a much more rigorous treatment than would be normally encountered in either commercial canning or in the poorest of home-cooking methods. The fact that the autoclaved proteins were less available to enzyme digestion

and had a lower biologic value than the raw pork proteins raises questions of considerable interest from the academic standpoint alone which need further investigation as to the cause and the exact result. The mildness of canning and home-cooking procedures strongly suggests that any changes occurring in these processes would be much less than in the rigorous autoclaving of the pork samples in this experiment. However, there is no way to know whether such changes would be insignificant without careful investigation. A problem of this nature to study the changes occurring in normal cooking of meats might easily involve such minute alterations as to be undetectable in growth experiments with young animals, and appear only in studies through growth to maturity followed by the additional stress of pregnancy and lactation.

METABOLIC ABNORMALITIES IN PATIENTS WITH CANCER OF THE GASTROINTESTINAL TRACT

Cancer of the gastrointestinal tract is so regularly associated with undernutrition that studies pointing out the existence of metabolic defects are of special interest. Since 1941 contributions by the group at the Memorial Hospital in New York have demonstrated a number of metabolic abnormalities in these patients. J. C. Abels, A. T. Gorham, G. T. Pack, and C. P. Rhoads (*J. Clin. Invest.* **20,** *749 (1941)*), found that plasma levels of vitamin A were reduced in patients suffering with gastrointestinal cancer. The low plasma levels in some patients were thought to be due to dietary deficiency or impaired absorption of the vitamin while in others they were perhaps better accounted for by an hepatic dysfunction. The observation was made, however, that the administration of yeast and lipocaic (containing no carotenoids) increased the plasma level of vitamin A. This, of course, may have been due to the lipotropic nature of these substances or perhaps to increased absorption as suggested by the work of I. L. Chaikoff, C. Entenman, and M. L. Montgomery (*J. Biol. Chem.* **168,** *177 (1947)*), who demonstrated that the action of pancreatic lipotropic substance is to enhance the availability of the methionine of ingested protein.

The hepatic function of patients with gastrointestinal tract cancer was studied by J. C. Abels *et al.* (*Ann. Int. Med.* **16,** *221 (1942)*). Measurements were made of blood prothrombin concentration, serum bilirubin, total serum protein and albumin and globulin fractionation, plasma vitamin A, urinary glucuronic acid excretion, red cell mean corpuscular volume, urinary urobilinogen, and cholesterol and cholesterol esters. Although one might not consider these tests the best to determine hepatic function, they were abnormal in a much higher percentage of patients with gastrointestinal tract cancer than in normal individuals, individuals with leukoplakia of the oral mucous membranes, or with atrophic gastritis.

Pursuing these metabolic abnormalities further, studies of protein metabolism were conducted. I. Ariel, P. E. Rekers, G. T. Pack, and C. P. Rhoads (*Ann. Surgery* **118,** *366 (1943)*) found that the serum proteins were generally reduced in patients with gastrointestinal tract cancer and that this was due chiefly to a fall in serum albumin concentration with no change or a slight rise in the serum globulin. There appeared to be no relation between previous dietary deficiency and the incidence of hypoproteinemia. The authors believed that the low serum albumin concentration might be related either to deficient absorption or to failure on the part of the liver to produce albumin. Glycine tolerance tests (I. Ariel, F. Jones, G. T. Pack, and C. P. Rhoads, *Ibid.* **117,** *740 (1943)*), demonstrated that the peak of absorption was considerably delayed in patients with gastrointestinal tract cancer as compared with normals. The

authors thus suggested that these two possible causes of hypoalbuminemia were probably operating concurrently. As further evidence for hepatic deficiency, J. C. Abels, C. W. Kupel, G. T. Pack, and C. P. Rhoads (*Cancer Research* **4,** *145* (*1944*)) demonstrated that the increased excretion of creatine and creatinine which was uniformly found following intravenous administration of glycine to normal subjects was not observed in 21 patients, 5 of whom had cirrhosis of the liver, and the remainder carcinoma of the gastrointestinal tract.

Additional studies on the defective plasma albumin synthesis in these patients did demonstrate, however, that despite a postoperative negative nitrogen balance the preoperative ingestion of high protein diets (from 2.18 to 2.68 g. of protein per kilogram of body weight) for from ten to twenty-two days prevented to a considerable extent the serious postoperative hypoproteinemia which had been observed. These results would appear to indicate that at least part of the low plasma albumin concentration observed in these patients was associated with a defective protein intake since it could be remedied by high preoperative protein feeding. In a subsequent publication J. C. Abels, I. M. Ariel, G. T. Pack, and C. P. Rhoads (*Proc. Soc. Exp. Biol. Med.* **56,** *62* (*1944*)) demonstrated that the fatty infiltration of the liver usually found at laparotomy in patients with gastrointestinal tract carcinoma could be diminished or made to disappear by the feeding of protein rich diets preoperatively. Continuing these studies, F. Homburger and N. F. Young (*Blood* **3,** *1460* (*1948*)) demonstrated that hypoproteinemia once established in patients with gastric cancer persists following operation despite administration of large amounts of protein. Ten patients with stomach cancer were observed. Eight were studied from seven to eighteen days, while for the remaining 2, the studies were continued for longer periods, that is twenty-six and one hundred and eight days, respectively. In addition, 3 patients with benign gastric ulcer were included in the series. All the patients with cancer were anemic. Immediately postoperative the patients were fed a casein hydrolysate as the sole source of nitrogen until it was possible to supplement with the usual gastrectomy hospital diet. Careful nitrogen balance determinations were made. Although in many instances considerable quantities of protein were retained, there were no significant changes in the total circulating plasma albumin in the patients with cancer. However, the 3 individuals with gastric ulcer did exhibit some rise in total circulating protein, which was quite marked in one. It should be noted, however, that one can hardly expect to replace the protein in a severely depleted individual in a few weeks. Thus in only one patient, the one continued for one hundred and eight days, is it possible to state that prolonged protein retention failed to induce an increase in total circulating proteins. Furthermore, it is difficult to determine when one has replaced the protein in a depleted individual. Return to normal body weight has been one of the few methods which has been used in the past as an indication of normal nutritional status. Unfortunately the authors do not include data on change in body weight nor the individuals' normal weights; rather they assumed that for each 30 g. of body protein stored, 1 g. of plasma albumin should have been formed. This figure was derived from the work of A. A. Weech, M. Wollstein, and E. Goettsch (*J. Clin. Invest.* **16,** *719* (*1937*)), but whether it can be applied directly to patients with cancer of the stomach, particularly those who are or have been anemic, is questionable. Certainly the formation of hemoglobin in these individuals would take precedence over the formation of plasma albumin (C. W. Heath and F. H. L. Taylor, *Ibid.* **15,** *411* (*1936*)). It is hoped that further studies of a long-term nature will be made in patients with gastric cancer to settle this problem.

The study of carbohydrate metabolism in patients with cancer of the gastrointestinal tract was initiated by I. M. Ariel *et al.* (*Ann.*

Int. Med. **20,** *570 (1944)*). Both patients with cancer and those with benign gastric lesions were found to have diminished concentrations of hepatic glycogen determined on specimens removed at laparotomy. Subsequently, J. C. Abels *et al.* (*Ibid.* **20,** *580 (1944)*), found that administration of glucose to patients with gastric cancer prior to laparotomy failed to increase the hepatic glycogen concentration in contrast to a definite increase in patients with benign gastrointestinal disorders. These observations were confirmed and extended by N. F. Young, J. C. Abels, and F. Homburger (*J. Clin. Invest.* **27,** *760 (1948)*). It was further observed that when adrenal cortical extract was administered intramuscularly together with the glucose by mouth the liver glycogen concentration of patients with gastric cancer did rise and was found to be within the range of the values obtained after glucose alone in the benign gastric lesions. Thus there appeared to be a significant increase in the deposition of liver glycogen following administration of glucose together with adrenal cortical extract in patients with gastric cancer. Similar studies with insulin instead of adrenal cortical hormone induced no increase in liver glycogen content. Studies of glucose absorption failed to reveal a significant depression of this function. Likewise the rate of glyconeogenesis as measured by urinary nitrogen excretion did not seem to be altered. The authors point out that the abnormality differs from that seen in Addison's disease or in adrenalectomized animals. In the former condition following the twelve hours' fast one would have expected a blood sugar and hepatic glycogen content lower than that found in the controls, that is the patients with benign gastric lesions. This was not the case. Thus the mechanism of the defective liver glycogen synthesis in gastric cancer was not elucidated. No mention is made in this study of the state of nutrition of the patients with gastric cancer or of those with benign gastric lesions. It might be suspected that those with cancer were considerably more undernourished than were the controls. If true, the apparent defect in adrenal cortical function might be on the basis of chronic severe undernutrition rather than related to the cancer *per se*.

The authors recommend on the basis of their study that administration of adrenal cortical extract as well as glucose prior to major surgical operation would appear to be desirable, especially to patients to be operated on for gastric cancer. This may well be the case but further evidence of beneficial results should be at hand before such a regimen is accepted as essential. Perhaps a preoperative feeding program of an intensive nature would diminish the incidence of these abnormalities by improving the patient's nutritional status. Certainly with respect to protein it has been shown by this group of workers that preoperative preparation with a high protein diet reduces the incidence of hepatic dysfunction and of postoperative hypoproteinemia.

The metabolic abnormalities found by these investigators in patients with cancer of the gastrointestinal tract should continue to be studied and extended to cancer in other sites. Since it is well known that undernutrition and malnutrition may lead to profound changes in digestion, absorption, and in intermediary metabolism, this important factor should be under careful scrutiny in future studies. In this regard, the necessity for developing better methods to measure accurately an individual's nutritional status is evident.

EFFECT OF CONVERSION PROCESSES ON THIAMINE CONTENT OF MILLED RICE

Parboiling or conversion processes applied to rice before milling enhance the nutritive value of the final product. An explanation for this effect has been sought. The con-

cept was held that parboiling probably causes redistribution of vitamins within the grain with movement from the outer layers where concentrations are relatively much greater into the inner portions of the grain. Some evidence had been presented to indicate that this treatment prevented complete removal of the germ (scutellum and embryo) by the milling process (*Nutrition Reviews* **6,** *35 (1948)*).

J. J. C. Hinton (*Nature* **162,** *913 (1948)*) examined the effects of the conversion process upon rice and reports the results of analyses demonstrating change in concentration of thiamine within the various constituent layers of the grain. In the raw grain most of the thiamine is shown to be in the germ, with greatest concentration in the scutellum which contains 45 to 50 per cent. In assessing the effects of conversion treatment, the Malek process was used. Small samples of rice were heated in water at 75° C. for four hours, partially dried on cloth, and steamed at a pressure of 15 pounds per square inch for fifteen minutes. Grains were dissected and the various layers were taken for analysis. These included the embryo, scutellum, pericarp, aleurone layer, and endosperm. Thiamine was determined by a microthiochrome method.

The process was demonstrated to effect a marked decrease in the thiamine content of the outer layers, the embryo, scutellum, and pericarp-aleurone, and an increase in concentration within the endosperm. Conditions of treatment were varied to determine the effects of single steps in the conversion procedure and of different conditions of treatment on redistribution and final concentration of thiamine.

Concentrations of thiamine within the grain varied with different conditions of steaming. When rice was contained in a beaker or on a watch glass, considerable water of condensation accumulated and the grains swelled and burst. Ninety-five per cent of the thiamine in the germ was lost. Forty-five per cent could be accounted for in the condensate. When steamed on filter paper, water of condensation was absorbed and there was much less loss of vitamin B_1 from the germ. The loss was even less when rice was placed in a cotton stoppered flask. It seemed, therefore, that great losses of thiamine resulted from excessive quantities of water of condensation about the grain. When steaming was omitted, redistribution of the vitamin with enrichment of deeper portions of the grain was observed, indicating that the additional heat of steaming alone had little or no effect on redistribution. It is concluded that redistribution is determined by entry into the grain of small quantities of water as the starch of the endosperm gelatinizes.

In an evaluation of the effect of conversion on retention of germ, residual germ was dissected from milled samples of raw and parboiled grain. There was some germ, mainly scutellum, on 90 per cent of grains of parboiled rice, and on only 6 per cent of the raw milled. Gelatinization of the endosperm occurring with conversion makes it more difficult to remove the germ. Raw milled rice containing germ was found to retain both embryo and scutellum, probably because these particular grains failed to be adequately polished in the milling process.

It is likely that other water soluble nutrients behave similarly under conversion treatment. The greater content of "B vitamins" in rice treated in this manner is the result, then, of redistribution of vitamins in the grain and adherence of a portion of the germ to the endosperm. From data presented by Hinton it is indicated that similar results might be obtained by simply soaking rice in water at 75° C. Light milling or less severe polishing of the grain might make possible retention of even greater quantities of germ, thereby further increasing the nutritive value of rice thus prepared.

NOTES

Protection Against Serum Hepatitis

With the extensive use of whole blood, plasma, and plasma fractions in the last few years, fear of transmission of hepatitis by transfusion has rapidly grown and is particularly warranted where large pools of plasma are used. Methods have been sought for inactivation of the virus without destroying the properties of the proteins in the solutions. S. S. Gellis *et al.* (*J. Clin. Invest.* **27,** *239* (*1948*)) using human volunteers demonstrated that the hepatitis virus may be inactivated in human albumin solution by heat. M. C. Blanchard *et al.* (*J. Am. Med. Assn.* **138,** *341* (*1948*)) have likewise shown that the irradiation of icterogenic serum and plasma by ultraviolet light is a practical, safe, and effective method for destroying the hepatitis virus.

The apparent inactivation of the hepatitis virus demonstrated in these studies, if it can be applied by hospitals and other processors of blood and blood products, should render plasma and its derivatives far safer than they are now. No method is yet available for destroying the hepatitis virus in whole blood and it is questionable whether practical means will be found but, of course, the likelihood of transmitting the hepatitis virus by transfusion of whole blood or unpooled serum or plasma is not nearly as great as when large pools are used.

Canadian Nutrition Bulletin

The Canadian Council on Nutrition has issued the first number of the *Canadian Bulletin on Nutrition*. It represents an additional effort to help people who are interested in nutrition to learn something of what is going on in the field of nutrition in different parts of Canada.

The first issue of the Bulletin deals with a variety of subjects including activities in nutrition in agriculture, fisheries, food processing, the Canadian Red Cross, and numerous other associations in Canada.

A charge of $1.00 is made for the Bulletin and it may be obtained by writing to the King's Printer, Ottawa, Canada.

Methionine In Liver Disease

Methionine has been used in experimental animals in the prevention and treatment of dietary hepatic injury. However, its virtue in this regard in man is yet to be conclusively demonstrated.

L. W. Kinsell *et al.* (*J. Clin. Invest.* **27,** *677* (*1948*)) studied in man the rate of removal of methionine administered intravenously from the blood and the subsequent excretion of methionine and sulfate sulfur in the urine. Normal individuals were compared with patients with hepatitis and with hepatic cirrhosis.

There seemed to be distinct differences between the normal individuals studied and those with liver disease with respect to the metabolism of the methionine administered intravenously. Although over 90 per cent of the fasting plasma l-methionine concentrations fell within the normal range, some were above normal, and a few were quite high, chiefly in very severe, active liver disease. After infusion of 50 ml. of a 3 per cent dl-methionine solution injected over a five minute period, peak blood values obtained fifteen minutes after the injection declined rapidly up to the thirty minute postinjection sample; thereafter the decline was slower up to one hundred and eighty minutes after the injection was given. There was evidence that the initial rapid fall was associated with diffusion of methionine out of the blood plasma but that the slower subsequent fall was more likely related either to the absorption of the amino acid into body tissue or actual utilization, either anabolic or catabolic. The rate of methionine removal from the plasma in acute hepatitis and in cirrhosis

was statistically slower than in the normal group. During convalescence from acute hepatitis the rate of disappearance became normal or, in some instances, greater than normal. Two of 3 individuals with chronic hepatitis had a normal rate of removal of plasma methionine after injection.

Urinary excretion of methionine and of sulfate following the administration of methionine intravenously to patients with liver disease differed little if any from that in the normal individuals.

Carbohydrates Labeled with Radioactive Carbon

Starch, glucose, fructose, and sucrose labeled with radioactive carbon are extremely valuable compounds for many phases of the investigation of intermediary carbohydrate metabolism. These compounds are difficult or impossible to synthesize by ordinary procedures *in vitro*.

E. W. Putman, W. Z. Hassid, G. Krotkov, and H. A. Barker (*J. Biol. Chem.* **173,** *785 (1948)*) described in detail procedures for isolation of these carbohydrates labeled with radioactive carbon during the process of photosynthesis in green leaves which had been exposed to an atmosphere of radioactive carbon dioxide in the presence of light. Turkish tobacco leaves were good starch producers for this purpose. The leaves were placed in water and kept in the dark for twenty-four hours to use up the reserve carbohydrates. Then they were allowed to photosynthesize for twenty-four hours in an atmosphere containing initially 10 to 15 per cent carbon dioxide tagged with radioactive carbon. After illumination for twenty-four hours, the leaves contained about 2.5 per cent starch, 0.65 per cent sucrose, and 0.85 per cent reducing sugars on a wet weight basis. The leaf was killed with boiling 80 per cent alcohol and the soluble sugars, glucose, fructose, and sucrose, extracted by refluxing in the same solution for six to eight hours. Most of the labeled glucose could be precipitated from this extract by the addition of inactive glucose. Labeled fructose was precipitated as the calcium-fructose complex from which it could be released readily. Starch labeled with radioactive carbon was prepared from the residue left after extraction with hot 80 per cent alcohol. If desired, additional labeled glucose could be prepared by hydrolysis of this starch.

Canna leaves produced little starch but were excellent producers of sucrose. When labeled sucrose was required, photosynthesis in Canna leaves was carried out at 17°C. for twenty-four hours in an atmosphere containing radioactive carbon dioxide. The leaves were extracted overnight with 80 per cent alcohol. On a wet weight basis of the fresh leaf, the final level of reducing sugars was 1.4 per cent and that of sucrose 1.8 per cent. The alcohol extract was diluted with water and the alcohol evaporated completely. The reducing sugars were removed from this solution by fermentation with Torula monosa which is unable to metabolize disaccharides. The labeled sucrose could then be readily isolated from the resulting solution without danger of contamination with reducing sugars.

Although purification of these labeled carbohydrates produced by photosynthesis was somewhat complex where a high degree of purity was required, this method of incorporating radioactive carbon into carbohydrates makes readily available relatively large amounts of these compounds and, in addition, the compounds have a relatively high specific radioactivity.

New Pamphlet

A pamphlet entitled "Your Teeth—How to Save Them" by Herbert Yahraes has been prepared in cooperation with the Council on Dental Health of the American Dental Association and the Dental Committee of the Health Education Service of the New York Tuberculosis and Health Association.

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* Representative of the public.
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The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress, and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

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